

GST-15 GCTGGCAGATCAGGCCAGAGCT

-195

GCTTCCCCGG

B-U B-M

GTTTTTTGG GTTTTTCGG

GATTTGGGAA AGAGGGAAAG GATTTGGGAA AGAGGGAAAG

GACCIGGGAA AGAGGGAAAG

CCCCGCTGGG TTTTGTTGGG TTTCGTTGGG

ACTCCCTAGG ATTTTTAGG ATTTTTAGG

CTGTCTGTTT

CCTCTTCCTG

rccrcccgr TTGTTGTGGT TCGTTGCGGT

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TTTTTTTG TTTTTTTG

TTGTTTGTTT

TTGTTTGTTT

	•	•		
	-42 TGTGAAGCGG TGTGAAGTGG TGTGAAGCGG	(p) -32 TGCGCACACT TGTGTATATT TGCGTATATT	TGYGTATATT	8 [°] 7
ncer	TCCCTGCCC TTTTTGTTT TTTTTTGTTT	GTA-GC (p) 33 -3 GCCAGTTCGC TGC GTTAGTTTGT TGT GTTAGTTCGT TGC	GTTAGTTYGT	
in Prostate Ca	TCCACCCCTC TTTATTTTT TTTATTTTTT	GTA-GC (p) -38-37 -36-35-34 -33 -32 CCCCGCGAI GICCCGGCGC GCCAGIICGC IGCGCACACI TITIGGAI GIIIGGIGI GIIAGIIIGI IGCGIAIAII	TTTCGC> <i>CGPS-6</i> YGGYGY TTTCGCGAT GTTTYGGCGC>	
Upstream Region of Differential Methylation in Prostate Cancer	-42 ATAAAGCAAT TICCTITCCI CIAAGCGGCC ICCACCCCIC ICCCCIGCCC IGIGAAGCGG ATAAAGTAAT TITITITIT ITAAGTGGIT ITTAITITIT ITTITIGITI IGIGAAGTGG ATAAAGTAAT ITTITITITI ITAAGCGGIT ITTAITITITI ITTITITITI IGIGAAGCGG		YGGTTTT AGGGAATTTT TTTTCGC>CGPS-6 YGGYGY GTTAGTTYGT TGYGTATATT CGPS-11 GGGAATTTT TTTTCGCGAT GTTTYGGCGC>	-29
n of Differentia	TTCCTTTCCT TTTTTTTTT TTTTTTTTT	-39 CAGCGGTCTT AGGGAATTTC TAGTGGTTTT AGGGAATTTT	AGGGAATTTT GGGAATTTT	
pstream Regio	ATAAAGCAAT ATAAAGTAAT ATAAAGTAAT	-39 CAGCGGTCTT TAGTGGTTTT TAGCGGTTTT		
Ţ	агааалгааа атаааатааа агаааатааа атаааатааа атаааатааа	-41 -40 srgrgcaagc rccgggarcg srgrgaagr trrgggarrg srgrgaagr trcgggarcg	CGPS-5	
Figure 2	ATAAAATAAA ATAAAATAAA ATAAAATAAA	GTGTGCAAGC GTGTGTAAGT GTGTGTAAGT		-31 -30
		SUE	BSTITUTI	E SHEET

B-U B-K

B-U B-M

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B-U B-W CAGCGCCGC CGGGGCTGGG TGGGGTTGGG CGGGGTTGGG -18 -17 -16 TAGTGGTTGT TAGCGGTCGT recesceac scccessers TGTGGTTGAT GTTTGGGGTG TGCGGTCGAC GTTCGGGGTG -22 -21 -20 -19 GGCGCCCTC GGTGTTTTT GGCGTTTTT GGGACTCCAG GGGATTTAG GGGATTTTAG ccascrecec secsacrecs TTAGTTGCGC GGCGATTTCG GGTGATTTTG -24 -27-26-25 TTAGTTGTGT

< GCTG CAARCCCCAC AICRCCARCA RCCCCA CGPS-8 SCCARCA GCCCCAACCC <6 CCGCTAAAGC CCCTAAAATC CCRCAAAA CGPS-12</p> <GCG CCRCTAAARC CCCTAAAATC CCRC CGPS-7</pre>

T (Rule 26) (RO/AU)

Figure 2 (Continued)

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	-35	B-U	B-M
-5	GCGGGACCAC	GTGGGATTAT	GCGGGATTAT
9- 4-	GCGGAGCGGG	GTGGAGTGGG	GCGGAGCGGG
	SCCGGCGGA GICCGCGGGA CCCICCAGAA GAGCGGCCGG CGCCGIGACI CAGCACIGGG GCGGAGCGGG GCGGACCAC	STIGGIGGGA GITIGIGGGA TITITIAGAA GAGIGGIIGG IGTIGIGATI TAGTATIGGG GIGGAGIGGG GIGGGATTAT B-U	STCGGCGGA GITCGCGGGA TITITIAGAA GAGCGGTCGG CGTCGIGAIT IAGIATIGGG GCGGAGCGGG GCGGGATIAI B-M
8-6-	CGCCGTGACT	TGTTGTGATT	CGTCGTGATT
-11 -10 -9 -8	GAGCGGCCGG	GAGTGGTTGG	GAGCGGTCGG
	CCCTCCAGAA	TTTTTAGAA	TTTTTAGAA
-13-12	GTCCGCGGGA	GTTTGTGGGA	GIICGCGGGA
-15 -14	GCCGGCGGGA	GTTGGTGGGA	GTCGGCGGGA

CGPS-9 <GCCGCCCT CAARCRCCCT AAAAAATCTT CTC</p> CAA CAGCCRCCCT

+46 B-U TCGCCGCCGC AGTCTTCGCC ACCAGTGAGT ACGCGCGGCC TIGITGITGI AGITITIGIT ATTAGIGAGI ATGIGIGGII ATTAGTGAGT ACGCGCGGTT 5 6 7 TCGTCGTCGT AGTTTTCGTT CGCTGGAGTT TGTTGGAGTT CGTTGGAGTT GTGAGGTTTT GCGAGGCCTT GCGAGGTTTT 7 CTCGGAGGCC TTCGGAGGTC TTTGGAGGTT ကူ びし TTTATAAGG CCTTATAAGG TTTATAGG

GCGAGGTTTT CGTTGGAGTT TCGTCGTC> CGPS-2 CGTT ATTAGTGAGT ACGCGCGGTT U CGPS-1

TTAA B-U CCAA +90 TTAA CAGCATGGGG TAGTATGGGG TAGTATGGGG TTAGAGTTTT TCAGAGCTCC TTAGAGTTTT CGCGTCCCC GGGATGGGGC GGGATGGGGT GGGATGGGGT CGCGTTTTCG 10 TGTGTTTTG ത

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占 3+3 +++ +++ # GST-Pi Gene ++++ ++++ ++++ ### +++ ++++ 3+4 XC ++ +++ ‡ +++ +++ **+ +** +++ 2+2 ‡ +++ +++ +++ **‡** X # ‡ ‡ ‡ + + ‡ ++ ‡ ‡ ‡ ‡ + ‡ æ Ø the 2+3 ပ္ပ ++ ‡ ‡ ‡ ‡ ‡ ‡ + ‡ ‡ m ‡ ‡ ‡ # # # # ‡ # ‡ + m + + + ij. 3+3 ‡ +++ ‡ ‡ ‡ ‡ ‡ ++ # ‡ # ‡ ‡ # ‡ ‡ ‡ B m + Sites 4+4 Methylation Status of Individual S S (HO/AU 2AN SUBSTITUTE SHIFET (Rule S ₹ ‡ # ‡ ‡ # ‡ # # ‡ # # ‡ ‡ ‡ ‡ ‡ m m E2 ⊠ (‡) + + # ‡ ‡ ‡ # ‡ ++ 11 ++ ‡ ‡ + + + + + + + + <u>PC3</u> # ‡ ‡ # # # ‡ # # # # # ‡ # ‡ + + + 呂 ‡ ### ‡ ## ‡ ## ‡ ‡ ‡ ## ‡ ## ‡ ## ‡ ‡ ‡‡ Ľ 3A Figure site 282 6 တ္

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PCT/AU99/00306 Received 30 June 1999

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Heart 3 ‡ Pancreas 9 # # # ‡ тапом Вопе (5) #### ‡ $\sharp | \sharp |$ ‡ Lung 3 ‡ ‡ # ## ‡ # Smooth muscle 9 ‡ ∄∄∄ ‡ Liver 9 ‡ **‡** Spleen 9 #### ‡ # # ‡ # Brain 9 ‡ # # # Blood (13)∄∄∄ ‡ ‡ ‡ ## ‡ ‡ ‡ ‡ (10)2 ###### ‡ ### ## ##### ပ္ပ (4) ## #### ‡ ‡ # # #### # # C(## BC6 #### ‡ ## ‡ ‡ # ‡ ‡ 0 #### まままま ### ‡ ### ### ‡ # PC-3 (10)‡ # ‡ # NormalP rostate (15) ‡ # # # # ‡ Site

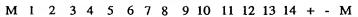
Figure 3B Methylation Status of Individual Sites in the GST-Pi Gene

09/673448 PCT/AU99/00306 Received 30 June 1999

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FIGURE 3B (cont'd)







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M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 ± - M



FIGURE 4A

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Sample Number

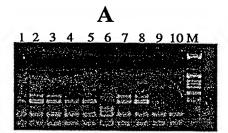
1 2 3 4 5 6 7 8 9 10 Mncncncncncncncncnc+-





Sample	Tissue	Gleason	% Methylation Non CG rich PCR
1	Normal	N/A	-
	Cancer	3+3	- - - - -
2	Normal	N/A	-
	Cancer	3+5	++
3	Normal	N/A	-
	Cancer	3+3	++-
4	Normal	N/A	-
	Cancer	3+5	-
5	Normal	N/A	_
	Cancer	2+2	++
6	Normal	N/A	-
	Cancer	3+3	-
7	Normal	N/A	-
	Cancer	2+3	++
8	Normal	N/A	-
	Cancer	3+3	+-+
9	Normal	N/A	- .
	Cancer	2+3	-{{}-
10	Normal	N/A	_
	Cancer	?	++

FIGURE 4B



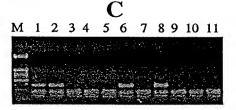


FIGURE 4C

Figure 5

M L D P N N C C C N N N -

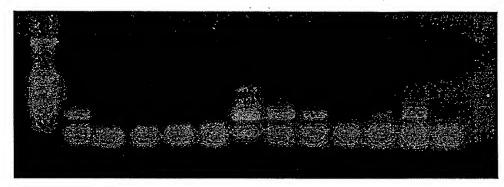
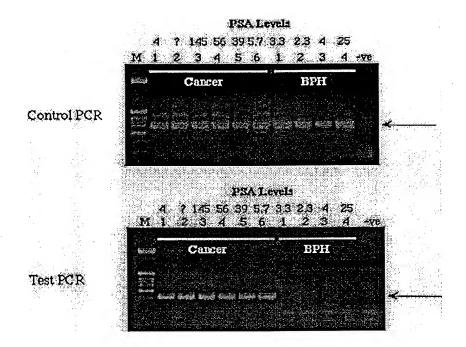


Figure 6



the state of the s

Cells added 0 2 20 100 1000 2000

Control PCR

CG-PCR

Secret Se

Figure 7B

Incubation Time Hours

1 2 4 6 o/n c 10 20 10 20 10 20 10 20 c

Incubation Time Hours

1 2 4 6 o/n c 10 20 10 20 10 20 10 20 10 20 c

Control PCR

CG-PCR

Control

Spring

Sprin

Spring

Spring

Spring

Spring

Spring

Spring

Spring

Spring

4°C

Room Temp

Figure 8

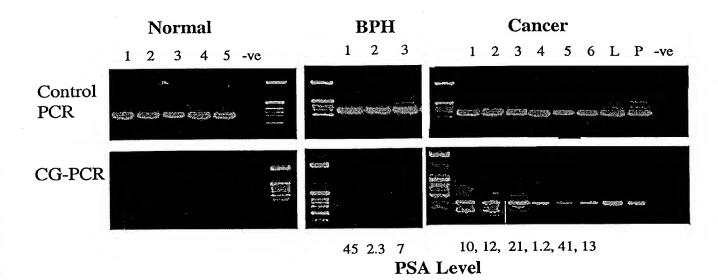


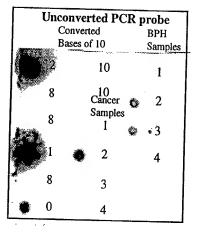
Figure 9

Liver Cancer Tissue DNA extracts



Figure 10

Test Oligo's



	Cor	verted PCR	
]		onverted ases of 10	BPH
	~		Samples
	2	10	* 1
	8	10 ancer	2
	8	Samples 1	
			3
*	1	2	4
	8	3	
₩	0	4	

Control Oligo

****		Converted Bases of 10	BPH Samples
	2	10	1
	8	Cancer	2
8	8	Samples 1	3
	1	2	4
	8	3	
*	0	4	
			ľ